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Original Research

Quantitative GC-FID analysis of heroin for seized drugs

Determination of heroin in seized drugs

Bayram Yüksel[1,2]
[1]Department of Forensic and Investigative Science, West Virginia University, Morgantown, WV, USA
[2]Giresun University, Espiye Vocational School, Giresun, Turkey

Abstract

Aim: Heroin is a semi-synthetic opioid, which is widely abused due to its euphoric effects. It is responsible for numerous deaths or diseases each year throughout the world. The goal of this work was to validate and establish a simple and reliable GC-FID method for quantitative analysis of heroin in seized drug samples in accordance with the predicted sample matrix. Material and Method: Detection parameters and chromatographic conditions were optimized in order to achieve an advanced method. Separation was accomplished on a HP-5 column (30 m-0.32 nm ID-0.25 µm) utilizing n-tetracosane as an internal standard at the concentration of 0.25 mg/mL in chloroform/methanol (1:1) mixture. Method validation was processed by means of specificity, linearity, accuracy, precision, range, quantitation limit and detection limit. Results: Method provided a great linearity with correlation coefficients (r^2=0.9994) for heroin. The limit of detection and limit of quantification values of GC-FID method for heroin analysis were 2.20 µg/mL and 7.33 µg/mL, respectively while the limit of linearity was 1000 µg/mL. Mean recovery value obtained from spike study was 99.89%, and relative error calculated after CRM analysis was equal to 1.80%, indicating that the method was accurate. Discussion: Inter-day stability of the instrument was demonstrated by use of the control chart. The method represented is comparatively simple, fast, precise, and pertinent for clandestine drug analysis in toxicological, pharmaceutical, and forensic laboratories.

Keywords

Drug Abuse; Clandestine Drug; Heroin; Toxicology; Forensic Chemistry; Validation; GC-FID

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Corresponding Author: Bayram Yüksel, Department of Forensic and Investigative Science, West Virginia University, Morgantown, WV, USA. Giresun University, Espiye Vocational School, Giresun, Turkey. T.: +1-304-276-6820, +90-454-310-1430 E-Mail: bayramyuksel83@gmail.com, bayram.yuksel@mail.wvu.edu
ORCID ID: https://orcid.org/0000-0001-7686-8648

Introduction

Heroin is a semi-synthetic drug formulated by acetylation of morphine presenting as the main opiate in opium poppy tears. As an intravenous illicit drug, heroin addiction is a phenomenon involving all age groups in Europe, and it is responsible for many deaths or diseases each year throughout the world [1,2]. Although some individuals may use heroin in a controlled manner [3], most of those who try to use heroin become addictive to this substance [4]. Chemical structure of heroin, 6-monoacetylmorphine (6-MAM) and morphine, are shown in Figure 1.

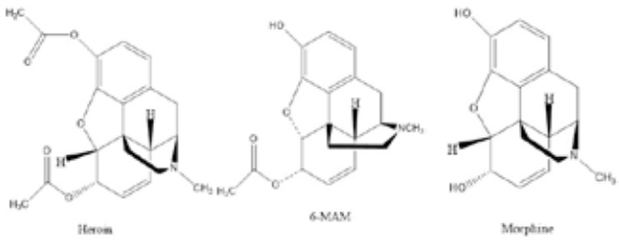

Figure 1. Chemical structure of: (a) heroin; (b) 6-MAM; (c) morphine

Following oral administration, heroin is subjected to comprehensive pre-systemic biotransformation by deacetylation, resulting in a pharmacologically active drug for the systemic delivery of morphine [5]. Although injection of heroin bypasses of pre-systemic metabolism effect, it can pass the blood-brain barrier quickly by reason of the acetyl groups causing it further lipophilic than morphine [6,7]. Nevertheless, heroin is rapidly metabolized first to 6-MAM following systemic administration, and the half-life of heroin in humans is nearly 1.8 to 7.8 minutes [8]. Like other opioids, morphine also causes euphoric, analgesic, and anxiolytic effects that is liable for its addictive function. The μ-opioid receptor in the brain intermediates to these effects of heroin [9]. However, heroin itself shows a comparatively small affinity for the μ receptor [10].

Heroin is converted to morphine through the 6-MAM in the body within minutes. In cases, morphine is usually a dominant active metabolite and is excreted from the body by transformation 3- and 6-glucuronides through the urine and bile. The existence of 6-MAM in urine differentiates the use of heroin from morphine. Low amount of codeine can also be involved in the urine of abusers by reason of acetyl codeine in heroin [11].

Heroin arrives Europe through four major transportation ways. The two essential directions are the Balkan and southern routes. The Balkan route passes Turkey through Bulgaria, Greece or Romania as well as the central, southern and Western Europe. Furthermore, Syria and Iraq have also appeared as an outgrowth of the Balkan route. The southern route which involves transition directions from Pakistan and Iran through African countries or directly into Europe by air or sea has recently been used. Northern route and the southern Caucasus crossing the Black Sea are the remaining minor routes [12]. Based on the Turkey Drug Report (2018): toxicological examination was accessible for the entire approved drug-related deaths. More than half of expirations was associated with multi-substances involving opioids, particularly heroin, engaged in approximately one-third of the deaths. The statistic of opioid-linked deaths recorded in Tukey has been approximately steady from 2014 up to now. Moreover, 8179 heroin cases were reported in 2016, and 5585.1 kg of heroin were seized in Turkey.

In the last decades, many chromatographic assays were advanced to determine heroin concentration in seized drugs as well as biological samples for toxicological, clinical, pharmaceutical, and forensic purposes [13-17]. As an alternative to immunoassays, thin-layer chromatography (TLC) appears as a beneficial method by reason of it is one of the practical and the cheapest techniques. Yet, such screening methods can fail to detect low concentration of drugs. For this reason, mass spectroscopy (MS) connected to liquid chromatography (LC) and gas chromatography (GC) are good instrumental examples for quantification and confirmation [18,19].

The aim of this research essentially focuses on validation and development of a GC-FID system for quantitative determination of heroin in clandestine drug specimens in order to improve the criminal justice system by providing enhanced objective conclusions. There are numerous works on this topic. However, major significance and novelty of this paper fundamentally depends on the advanced chromatographic resolution and detection sensitivity throughout validation and optimization of the technique designed in accordance with the predicted sample matrix. Ultimately, the study showed a satisfactory separation of all analyte peaks within 13 minutes.

Material and Methods

Instrumentation

The analysis was performed using Agilent GC 6890N (Santa Clara, California, USA) equipped with a flame ionization detector (FID) and an automated liquid sampler. An Agilent 7683 Series Auto-Injector was utilized for the injection of samples. This instrumentation was utilized for validation and optimization of an analytical method based on the determination of heroin in illicit samples.

Standard Solutions and Reagents

Stock solutions of methanol, chloroform, and n-tetracosane powder were obtained from Merck (Darmstadt, Germany). Certified reference material (CRM) of heroin, and caffeine, codeine, morphine, and 6-MAM solutions were obtained from Lipomed Services to Health®, Switzerland. All the other solvents and chemicals used during laboratory work were of analytical reagent grade. Ultrapure water (Merck Millipore Direct-Q8, Germany) with a resistivity of 18MΩ.cm, was used to prepare the solutions during the experimental process.

Sample Preparation and Procedure

An Agilent Model 6890N gas chromatograph was utilized during analyses. One mL of the prepared solutions was placed into an autosampler vial for analysis, and separation was achieved on an HP-5 column (30 m, 0.32 mm ID, 0.25 μm) using an IS (tetracosan at the concentration of 0.25 mg/mL) in chloroform/methanol (1:1, v/v) mixture. Ultrahigh purity (99.999 percent) hydrogen was chosen as the carrier gas with a flow rate of 1.5 mL/minute. The flame ionization detector and the injection port were sustained at 280 °C. An Agilent 7683 Series Auto-Injector was used during injection of samples. In the splitless mode (20:1), 2 mL amounts of samples were injected. Then, isothermally programmed oven temperature was adjusted to 180 °C for 10.00 minutes, and nitrogen was utilized as the auxiliary make-up gas for the detector. Operating parameters of the GC-FID system for heroin analysis was given in Table 1.

All samples and calibration standard solutions were prepared by use of an appropriate amount of our working solution: chloroform/methanol (1:1, v/v) mixture containing the IS (tetracosane at the concentration of 250 μg/mL). In order to prepare the

Table 1. Operation conditions for heroin analysis by GC-FID

Column	30 m – 0.32 mm ID – 0.25 mm HP-5	
Injection	Splitless: 1/20	
Injector Temperature	285 °C	
Carrier Gas	Hydrogen at 1.5 mL/min flow rate	
Oven Temperature Ramp Program	Initial Temperature	180 °C
	Start Time	1 minute
	Temperature Rate	10 °C/min
	Final Temperature	280 °C
	Final Time	10 minutes
Detector Temperature	275°C	
Analysis Time	13.0 minutes	

calibration standards at the concentrations of 0, 50, 100, 250, 500 and 1000 µg/mL, heroin powder standards were diluted in our working solution described above. The solution was kept at 4±1°C when not in use and warmed to room temperature before use.

Method Validation

As outlined in International Conference on Harmonization (ICH) guidelines, this GC-FID method for analysis of heroin content in illicit specimens was validated based on specificity, accuracy, precision, range, linearity, quantitation limit and detection limit.

Results

Calibration

All calibration standards were analyzed 5 times, and a calibration curve of area heroin/area tetracosane versus concentration of heroin standards was drawn (Figure 2). The correlation coefficient (r^2) and equation of the calibration curve for heroin were respectively found to be r^2=0.9994 and y=2.1106x-0.0003 where y stands for area heroin/area tetracosane, and x is the heroin concentration in µg/mL. The chromatogram of heroin standard at 600 µg/mL, obtained after analysis according to proposed GC-FID method, was illustrated in Figure 3.

Limit of Detection, Quantification, and Linearity

The limit of detection (LOD) and lowest limit of quantification (LOQ) were determined by means of the standard deviation of the response and the slope of the calibration curve, according to International Conference on Harmonization (ICH) guidelines, LOD=3.3σ/S, LOQ=10σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve. The LOD and LOQ values of GC-FID method for heroin analysis were 2.20 µg /mL and 7.33 µg /mL, respectively. Limit of linearity (LOL) is the concentration at which the calibration curve departs from the linearity. Dynamic range refers to concentration intervals from LOQ to LOL, which was found between 7.73 µg/mL and 1000 µg/mL, in this study.

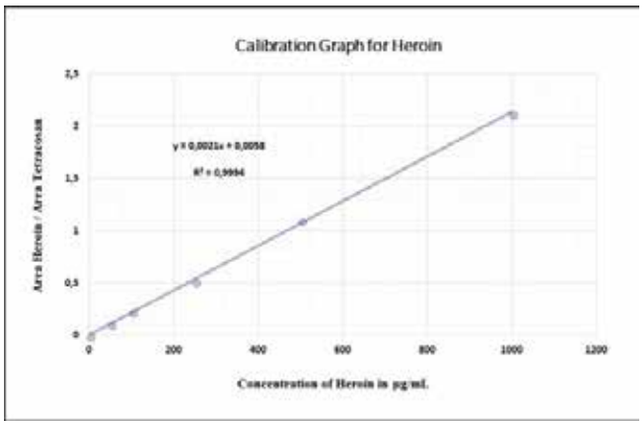

Figure 2. Calibration graph of heroin, constructed after GC-FID analyses. Concentration of heroin is given in µg/mL.

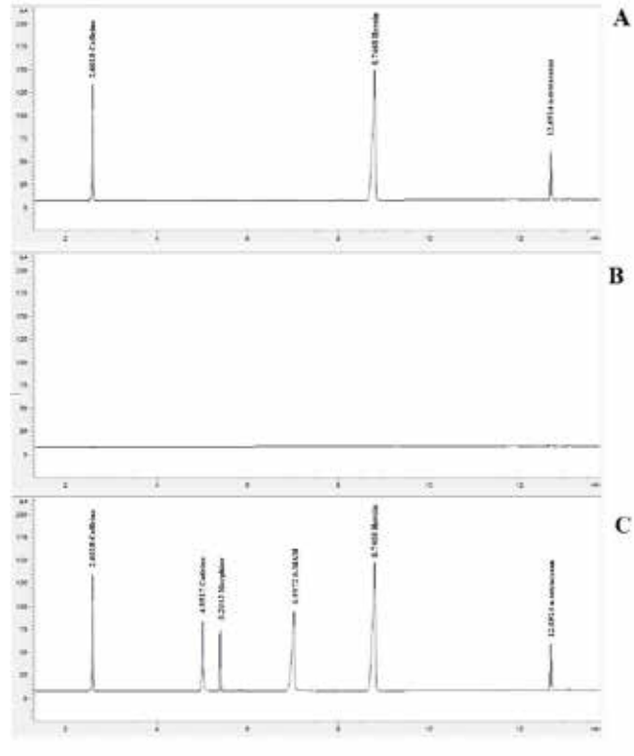

Figure 3. The chromatograph of (A) heroin standard injection; (B) placebo and working solution; (C) sample chromatogram, obtained after the analysis according to proposed GC-FID method

Precision

The precision of the method was assessed by means of repeatability, intermediate precision, and reproducibility parameters. The reproducibility of the recommended method was characterized by analysis of six different samples at the same concentration of 700 µg/mL from the same certified reference solution of heroin. Caffeine concentration of these samples was adjusted as 300 µg/mL to make sure the matrix effect. The mean of measured heroin concentrations was found as 699.17±9.64 µg/mL with 1.38% relative standard deviation (RSD). The result of the reproducibility study was shown in Table 2. Repeatability was controlled by injecting six individual samples of heroin at 200 µg/mL concentration while the intermediate precision was assessed by two analysts. Mean heroin concentrations from Analyst-A and Analyst-B were calculated as 202.17±2.56 µg/mL and 201.33±2.50 µg/mL, respectively. Repeatability study was summarized in Table 3.

Control Chart

The control chart study provides observation of inter-day and intra-day differences in peak intensity. From this point of view, a convenient procedure for monitoring the inter-day stability of the instrument was verified by use of the control chart. A mixture solution containing heroin at 500 µg/mL and caffeine at 500 µg/mL concentration was analyzed by GC-FID method once a month during a year, and the mean concentration of heroin was found as 499.330±10.30 µg/mL. After that, warning limits were calculated from the following formula: Warning

Table 2. The results demonstrating the reproducibility of the method for precision study.

Sample	Measured Heroin Concentration (µg/mL)
1	694
2	692
3	714
4	702
5	705
6	688
Statistics	
Mean±SD	699.17±9.64
RSD%	1.38

SD: Standard deviation, RSD: relative standard deviation

Table 3. The results illustrating the repeatability and intermediate precision. (Six individual heroin samples at the concentration of 200 µg/mL were analyzed by two analysts)

Heroin Sample	Analyst-A (µg/mL)	Analyst-B (µg/mL)
1	205	199
2	200	204
3	203	201
4	199	198
5	205	204
6	201	202
Statistics		
Mean±SD	202.14±2.56	201.33±2.50
RSD%	1.26	1.24

SD: Standard deviation, RSD: relative standard deviation

Limits = x_{mean}±2σ. Lowest warning limit (LWL) and upper warning limit (UWL) were equal to 478.73 µg/mL and 519.93 µg/mL, respectively. Similarly, control limits were calculated from the formula: Control Limits = xmean± 3 σ. Lowest control limit (LCL) and upper control limit (UCL) were equal to 468.43 µg/mL and 530.23 µg/mL, respectively. The control chart study was demonstrated in Figure 4.

Discussion

Optimization

Several essential parameters were improved in order to accomplish the most excellent performance from this chromatographic study. The main principle was based on a choice of the convenient column, deciding the best oven temperature program, selection of a suitable internal standard (IS), the election of concentration range due to heroin concentration in illicit drug specimens, and building the linearity. From this perspective, preliminary investigations were performed for arrangement and decision of the chromatographic conditions for the GC-FID determination of heroin.

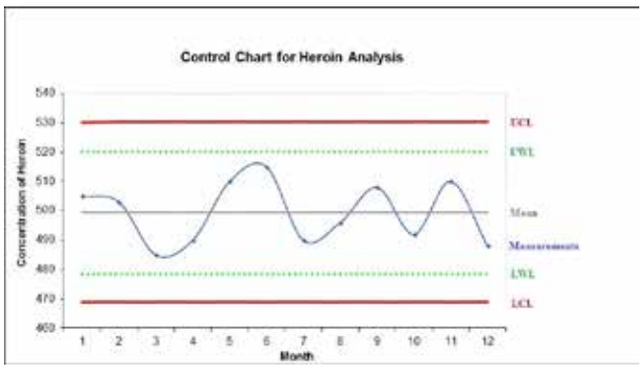

Figure 4. Control chart of heroin, performed by GC-FID. Concentration of heroin is given in µg/mL while UCL, UWL, LWL and LCL stand for upper control limit, upper warning limit, lowest warning limit and lowest control limit, respectively. The blue line represents the stability in the heroine concentrations measured over

As the column shows a critical role in an improved chromatographic separation [20], the selection of the favorable capillary column was decided on the basis of four necessary items: column length, stationary phase, film thickness, and column I.D. [19]. According to Tony Taylor's 2015 paper, temperature has an effect on retention and relative retention in GC. Once the temperature is varied, the selectivity of the separation is also changed [21]. In order to obtain the excellent chromatographic resolution and detection sensitivity, an outcome of the oven temperature rate was analyzed by examining not only various oven temperature programs but also ramp rates up to highest oven temperature of 300 °C. Hence, 280 °C was decided as the final temperature. Then, the gradient temperature program in the injection and the GC oven were examined. Subsequently, the improved program provided an adequate separation of entire analyte peaks within 13 minutes. Since utilizing of IS is suggested to avoid potential miscalculations following injection of different sample volumes in the chromatographic equipment [18], n-tetracosane was therefore chosen as IS. Selection of a favorable solvent is important to provide that both the IS and the target analyte are completely dissolved. Therefore, chloroform/methanol mixture (1:1, v/v) was chosen as a solvent.

Selectivity/ Specificity

Selectivity/Specificity of an analytical assay can be characterized as the detection ability of the desired component in the existence of alternative analytes which can be supposed to be present in a complex matrix such as impurities, degradation products, and diluents [19,21]. The components in the seized illicit drug are generally country dependent. For this reason, a method must either be validated or revised according to the predicted sample matrix [14]. To appraise the specificity of this chromatographic method, our working solution: chloroform/methanol (1:1, v/v) mixture and placebo solution containing the IS (n-tetracosane at the concentration of 0.25 mg/mL) without the heroin were injected into the GC-FID system. The specificity of the method was carried out in existence of our working solution and placebo, which has heroin free solution. A characteristic chromatogram of heroin standard solution is presented in Figure 3(A). As can be observed in Figure 3(B), there is no peak correlated to placebo or dilution solution was identified at the retention time of heroin. Figure 3(C) demonstrates the sample chromatogram in which heroin was mixed with compounds (caffeine, codeine, morphine, and 6-MAM) usually encountered in real cases. Satisfactory selectivity was verified for heroin and IS by investigating whether all these analytes were separated adequately from one another on a chromatogram.

Accuracy

Accuracy can be described as the proximity of a quantified magnitude to an approved certified value or known value [19]. The accuracy of the assay was evaluated by means of the percentage of recovery data from spike analysis. According to our experience, heroin samples seized in Turkey generally contain caffeine. Reference caffeine and heroin solution were therefore spiked in three different amounts. The mean of recovery was found as 99.89% for heroin (see Table 4). In addition, relative

Table 4. Accuracy study for heroin analysis by GC-FID

Spiked Caffeine (µL)	Spiked Heroin (µL)	Theoretical Heroin Concentration (µg/mL)	Measured Heroin Concentration (µg/mL)	Recovery (% R)
100	900	900	894±6.0	99.33
350	650	650	662±5.0	101.85
800	200	200	197±4.0	98.50
Mean Recovery				99.89

Table 5. Assessment of Relative Error (RE) and Coefficient of Variation (CV). Before the analysis, 0.25 mg of n-tetracosan was dissolved in 1 mL amount of the heroin standard solution.

Standard Solution	Number of Analysis	Certified Value (µg/mL)	Measured Value (µg/mL)	CV (%)	RE (%)
Heroin (Sigma-Aldrich)	11	1.00±0.00	0.982±0.01	1.12	1.80

error (RE) and coefficient of variation (CV) were also used to assess the accuracy of the method (see Table 5).

Conclusion

Heroin addiction is still a global public health problem through new psychoactive substances such as synthetic cannabinoids have been widely used by abusers. Turkey is exposed to international illegal heroin trafficking as transit and/or destination country. Hence, a significant amount of heroin has been seized by law enforcement agencies, indicating the importance of forensic drug analysis. In this study, GC-FID method for heroin analysis in illicit drug samples was developed and validated for accuracy, precision, and linearity. The mean recoveries obtained from CRMs analysis were found as 99.89% with relative error equal to 1.8%, indicating the method was accurate. The method provided LOD and LOQ equal to 2.20 µg/mL and 7.73 µg/mL, respectively. The GC-FID method is relatively fast, simple, precise, and applicable for routine forensic and pharmaceutical analysis. This work will improve the criminal justice system by providing quantitative and objective conclusions while examiners are presenting forensic evidence in the court.

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Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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